Miltenyi Biotec

CD28 MicroBead Kit
human

Order no. 130-093-247

Contents

1. Description
   - 1.1 Principle of the MACS® Separation
   - 1.2 Background information
   - 1.3 Applications
   - 1.4 Reagent and instrument requirements
2. Protocol
   - 2.1 Sample preparation
   - 2.2 Magnetic labeling
   - 2.3 Magnetic separation
3. Example of a separation using the CD28 MicroBead Kit
4. References

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

1. Description

This product is for research use only.

Components	**0.2 mL CD28 Antibody, anti-human, PE:** monoclonal CD28 antibodies conjugated to R-phycoerythrin (PE) (isotype: mouse IgG1). **2 mL Anti-PE MicroBeads:** MicroBeads conjugated to monoclonal anti-PE antibody (isotype: mouse IgG1).
Capacity	For 10^9 total cells, up to 100 separations.
Product format	CD28 Antibody, anti-human, PE and Anti-PE MicroBeads are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 2 – 8 °C. Do not freeze. The expiration date is indicated on the vial label.

This product is applicable for the separation of cells from rhesus monkey *(Macaca mulatta)* and cynomolgus monkey *(Macaca fascicularis)*.

1.1 Principle of the MACS Separation

First, the $CD28^+$ cells are labeled with CD28 Antibody, anti-human, PE. Subsequently, the cells are magnetically labeled with Anti-PE MicroBeads. Then, the cell suspension is loaded onto a MACS Column which is placed in the magnetic field of a MACS Separator. The magnetically labeled $CD28^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of $CD28^+$ cells. After removing the column from the magnetic field, the magnetically retained $CD28^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background information

CD28 (formerly Tp44) is a type I transmembrane protein belonging to the Ig receptor superfamily. CD28 is found on most $CD4^+$ T cells, many $CD8^+$ T cells, and on malignant plasma cells (myeloma cells).[1–3] CD28 is expressed on naive and memory T cells but is downregulated at late stages of terminal effector T cell differentiation.
Ligation of CD28 with CD80 (B7-1) or CD86 (B7-2) on target cells provides a critical costimulatory signal for T cell activation.[4] Blockade of CD28-B7 interaction leads to an inhibition of immune responses both *in vivo* and *in vitro*.[5]

1.3 Applications

- Isolation of $CD28^+$ cells from peripheral blood mononuclear cells (PBMCs) for phenotypical or functional characterization.
- Depletion of $CD28^+$ cells from PBMCs.
- Isolation of $CD28^+$ T cell subsets by combining the CD28 MicroBead Kit with kits for the isolation of untouched T cells.

1.4 Reagent and instrument requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS™ Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C). Degas buffer before use, as air bubbles could block the column.

  ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

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- MACS Columns and MACS Separators: CD28$^+$ cells can be enriched by using MS, LS, or XS Columns or depleted with the use of LD, CS, or D Columns. Cells which strongly express the CD28 antigen can also be depleted using MS, LS, or XS Columns. Positive selection or depletion can also be performed by using the autoMACS Pro Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, SuperMACS II
LS	10^8	2×10^9	MidiMACS, QuadroMACS, SuperMACS II
XS	10^9	2×10^{10}	SuperMACS II
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, SuperMACS II
D	10^9		SuperMACS II
Positive selection or depletion			
autoMACS	2×10^8	4×10^9	autoMACS Pro

▲ **Note:** Column adapters are required to insert certain columns into SuperMACS™ II Separator. For details see the respective MACS Separator data sheet.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD3 Antibody, anti-human, FITC; CD4 Antibody, anti-human, FITC; or CD8 Antibody, anti-human, FITC. For more information about other fluorochrome-conjugates see www.miltenyibiotec.com.
- (Optional) CD8$^+$ T Cell Isolation Kit (# 130-096-495), CD4$^+$ T Cell Isolation Kit (# 130-096-533), or Pan T Cell Isolation Kit (# 130-096-535).
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells.
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.
- (Optional) Pre-Separation Filters (30 µm) (# 130-041-407) to remove cell clumps.

2. Protocol

2.1 Sample preparation

When working with anticoagulated peripheral blood or buffy coat, peripheral blood mononuclear cells (PBMCs) should be isolated by density gradient centrifugation, for example, using Ficoll-Paque™.

▲ **Note:** To remove platelets after density gradient separation, resuspend cell pellet in buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully aspirate supernatant. Repeat washing step.

For details refer to the protocols section at www.miltenyibiotec.com/protocols.

When working with tissues, prepare a single-cell suspension using the gentleMACS™ Dissociator.

For details refer to www.miltenyibiotec.com/gentlemacs.

▲ Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, we recommend using density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101).



2.2 Magnetic labeling

▲ Work fast, keep cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.

▲ Volumes for magnetic labeling given below are for up to 10^7 total cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^7 total cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ For optimal performance it is important to obtain a single-cell suspension before magnetic separation. Pass cells through 30 µm nylon mesh (Pre-Separation Filters (30 µm), # 130-041-407) to remove cell clumps which may clog the column. Wet filter with buffer before use.

▲ Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 48 µL of buffer per 10^7 total cells.
4. Add 2 µL of CD28 Antibody, anti-human, PE per 10^7 total cells.
5. (Optional) Add staining antibodies, e.g., CD8-FITC or CD8-APC according to manufacturer's recommendation.
6. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
7. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Resuspend cell pellet in 80 µL of buffer per 10^7 cells.
9. Add 20 µL of Anti-PE MicroBeads per 10^7 cells.
10. Mix well and incubate for an additional 15 minutes at 4–8 °C.
11. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
12. Resuspend up to 10^8 cells in 500 µL of buffer.

    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.

    ▲ **Note:** For depletion with LD Columns, resuspend up to 1.25×10^8 cells in 500 µL of buffer.
13. Proceed to magnetic separation (2.3).

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2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of $CD28^+$ cells. For details see table in section 1.4.

Magnetic separation with MS or LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details see the respective MACS Column data sheet.
2. Prepare column by rinsing with the appropriate amount of buffer:
   MS: 500 µL LS: 3 mL
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with the appropriate amount of buffer. Collect total effluent; this is the unlabeled cell fraction. Perform washing steps by adding buffer three times. Only add new buffer when the column reservoir is empty.
   MS: 3×500 µL LS: 3×3 mL
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette the appropriate amount of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
   MS: 1 mL LS: 5 mL
7. (Optional) To increase purity of $CD28^+$ cells, the eluted fraction can be enriched over a second MS or LS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

Magnetic separation with XS Columns

For instructions on the column assembly and the separation refer to the XS Column data sheet.

Depletion with LD Columns

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details see LD Column data sheet.
2. Prepare column by rinsing with 2 mL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 2×1 mL of buffer. Collect total effluent; this is the unlabeled cell fraction. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.

Depletion with CS Columns

1. Assemble CS Column and place it in the magnetic field of a suitable MACS Separator. For details see CS Column data sheet.
2. Prepare column by filling and rinsing with 60 mL of buffer. Attach a 22G flow resistor to the 3-way stopcock of the assembled column. For details see CS Column data sheet.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 30 mL buffer from the top. Collect total effluent; this is the unlabeled cell fraction.

Depletion with D Columns

For instructions on column assembly and separation refer to the D Column data sheet.

Magnetic separation with the autoMACS Pro Separator

▲ Refer to the respective user manual for instructions on how to use the autoMACS Pro Separator.

▲ Buffers used for operating the autoMACS Pro Separator should have a temperature of ≥ 10 °C.

Magnetic separation with the autoMACS Pro Separator

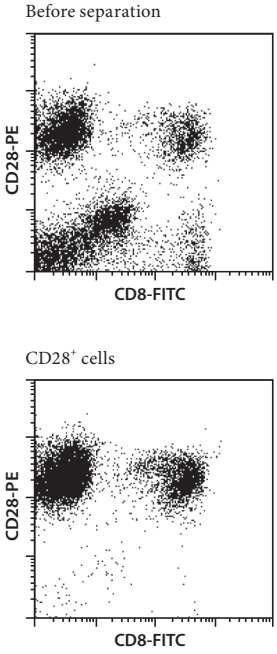
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and fraction collection tubes in rows B and C.
3. For a standard separation choose one of the following programs:

   Positive selection: "Possels"
   Collect positive fraction in row C of the tube rack.

   Depletion: "Depletes"
   Collect negative fraction in row B of the tube rack.

3. Example of a separation using the CD28 MicroBead Kit

$CD28^+$ cells were isolated from human PBMCs using the CD28 MicroBead Kit, an MS Column, and a MiniMACS™ Separator. Cells are fluorescently stained with CD8-FITC and CD28-PE. Cell debris and dead cells are excluded from the analysis based on scatter signals and PI fluorescence.



4. References

1. June, C. H. *et al.* (1990) Role of the CD28 receptor in T-cell activation. Immunol. Today 11: 211–216.
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4. Freeman, G. J. *et al.* (1993) Cloning of B7-2: a CTLA-4 counter-receptor that costimulates human T cell proliferation. Science 262: 909–911.
5. Linsley, P. S. and Ledbetter. J. A. (1993) The role of the CD28 receptor during T cell responses to antigen. Annu. Rev. Immunol. 11: 191–212.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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